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(54) **THE USE OF MONOMERIC/POLYMERIC MATERIALS IN THE PREPARATION OF A CURABLE COMPOSITION FOR CARTILAGE REPAIR**

Die Verwendung von monomerem/polymerem Material zur Herstellung einer härtbaren Zusammensetzung für die Reparatur von Knorpeln

L'utilisation de matériaux monomériques/polymériques pour préparer une composition durcissable destinée à la réparation de cartilage

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The file contains technical information submitted
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Description

[0001] This invention relates to the use of physiologically acceptable monomeric/polymeric materials in the preparation of a curable composition for cartilage repair by *in situ* curing in a human or animal body.

[0002] The use of physiologically acceptable polymeric materials in the preparation of biomedical appliances such as hearing aids, artificial eyes and dentures is well known, as is the use of polymeric materials as bone cements in the field of orthopaedics. Such polymeric materials are often used in the form of a curable composition which is initially in a fluid, semi-liquid, dough-like or other mouldable form, but which cures or hardens at the temperature of use to form a strong solid of physical properties dependent on the use to which it is being put. Examples of such curable compositions are to be found in International Patent Application No. WO89/03695 and GB Patent 2 107 341 which disclose the use of curable compositions comprising a powdered methacrylate polymer mixed with an acrylic monomer. For such applications as the preparation of hearing aids, artificial eyes and dentures which require dimensional accuracy, it is important to provide compositions of low linear curing shrinkage and GB Patent 2 107 341 is directed to such an aim. For use as bone cement it is important to prevent subsequent failure at the bone-cement interface and, to this end, WO89/03695 discloses the inclusion of a cell growth stimulant such as human growth hormone so as to increase the rate of healing of, for example, a bone fracture and give a joint of increased strength.

[0003] EP-A 088,845 discloses a mixture of a monomeric and polymeric acrylate or methacrylate, the monomeric component comprising a saturated oxygen-containing heterocyclic ester, especially tetrahydrofurfuryl acrylate or methacrylate, as having low shrinkage upon curing at room temperature, thus imparting dimensional stability. This polymeric material is proposed for "biomedical" use, e.g. in the aural, ophthalmic, surgical and dental fields, especially in the manufacture of hearing aids, dentures, tooth fillings and crowns. Use as a bone cement is also suggested.

[0004] EP-A 032 249 describes a mixture of tetrahydrofurfuryl methacrylate with polyethyl methacrylate as a dental resin for making a temporary, but tough and adherent crown-and bridge.

[0005] M. P. Patel and M. Braden, "Heterocyclic methacrylates for clinical applications", *Biomaterials* 12, 649-652 (September 1991), have described compositions of tetrahydrofurfuryl methacrylate and polyethyl methacrylate for producing low shrinkage materials. The implication of this paper is that these are for dental use and for the manufacture of hearing aids. The paper reports tests of the exothermicity of the curing reaction.

[0006] Surprisingly, it has been found that certain curable compositions comprising a methacrylate polymer mixed with an acrylate or methacrylate monomer containing a heterocyclic group (defined below), such as tetrahydrofurfuryl acrylate or methacrylate, can be employed to promote cartilage repair, with or without the addition of cell growth stimulants such as human growth hormone.

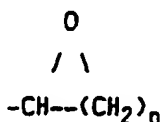
[0007] Cartilage, which differs in construction from bone, has previously been considered substantially non-repairable. Attempts to repair cartilage currently include the introduction of carbon fibres behind the cartilage; however such carbon fibres are brittle and, while a fibrous tissue forms in the implanted area, the resulting growth is not that of true cartilage composed of chondrocytes expressing their normal phenotype and producing their own matrix components. Implantation of cartilage components and hydrogel compositions have also been tried with limited success. There is therefore a great need for a breakthrough in the treatment of cartilage defects.

[0008] According to the present invention there is provided the use of a monomer/polymer mixture in the preparation of a curable composition for introduction at or adjacent to a cartilage requiring repair in a human or animal body to promote said repair by curing *in situ*, the monomer component being selected from monomeric esters of general formula I



wherein R is a hydrogen atom or a methyl group, m is 0, 1 or 2, and X is a 3 to 6 membered heterocyclic ring and the polymer component is selected from acrylate and methacrylate polymers and copolymers thereof. Preferably X is an oxygen-containing heterocycle.

[0009] The monomeric ester component is preferably selected from methacrylates where X is a heterocyclic group of formula



where n is 1, 2, 3 or 4. Tetrahydrofurfuryl methacrylate ($R=CH_2$, $m=1$, $n=3$) is particularly preferred. These monomers may be admixed with other monomers to control hydrophilicity, for example hydroxyethyl methacrylate to increase hydrophilicity or isobornyl methacrylate to decrease hydrophilicity. The polymer component is preferably a methacrylate polymer, preferably poly(ethyl methacrylate), but other polymers such as poly(methyl methacrylate), poly(hydroxyethyl methacrylate), or poly(tetrahydrofurfuryl methacrylate) may be employed as well as copolymers thereof. The copolymer component may be selected to control hydrophilicity.

[0010] The composition is suitably in the form of a mixture of finely divided solid polymer, suitably prepared by suspension polymerisation, in liquid monomer. The composition may include initially, or have added to it at the point of use, suitable activators for the curing such as free radical catalysts, e.g. peroxide/amine initiator systems. Alternatively, photoinitiators could be used, e.g. camphor quinone/tertiary amine systems well known in the art. Additional additives such as stabilisers and fillers and x-ray opacifying agents may be present. These include, for example, quinone type inhibitors in the monomer, and/or inorganic fillers to increase hardness and reduce polymerisation shrinkage. In particular, hydroxyapatite may be used for this purpose and to improve biocompatibility. In addition, antibiotic components such as gentamicin may be added to avoid infection. Other possible therapeutic additives include anti inflammatory drugs, hydrocortisones, dexamethasone and drugs for the treatment of osteoarthritis when promoting tissue repair in a diseased joint. Other examples are antifungal agents and antimicrobial agents. Other possible additives include porogens such as collagen or dextran to increase the porosity of the material or materials which function as protein carriers.

[0011] A particularly preferred additive is a cell growth stimulant such as those described in WO89/03695, and in particular, human growth hormone. Other growth factors such as TGF- β , IGF I, FDGF and FGF may be used.

[0012] The ratio of polymer to monomer component can vary dependent on the reaction time required and the consistency of composition required initially. Suitably the ratio of polymer to monomer is from 1:1 to 2:1 by weight, preferably 1.25:1 to 1.75:1. The curing should desirably occur at body temperature and curing is desirably effected over a period of 5 to 20 minutes, preferably 10 to 15 minutes. The use of such compositions as biomaterials in dental, aural and ophthalmic fields is described in GB 2 107 341.

[0013] It is postulated that the ability of such cured compositions to promote tissue repair results from their ability to absorb water to an extent which allows absorption of tissue fluid from the area requiring repair, while swelling in the tissue to provide good bonding conditions. It is postulated that, in order to be well suited for use in accordance with the present invention, the biopolymer composition when cured should have a water uptake in the region of 5 to 30% w/w over a period of six months to 2 years. It is also recognised that the low shrinkage properties of such biopolymers combined with the slight swelling occurring with water uptake give materials which bond firmly in use, for example in cartilage, and are not readily dislodged.

[0014] The monomer/polymer mixture is used to manufacture a composition intended for introduction at, or adjacent to damaged cartilage to promote cartilage repair. It has been found advantageous to apply the composition below, preferably slightly below the surface of the subchondral bone in order to optimize the formation of a cartilage layer, as a result of enhanced cell proliferation and differentiation.

[0015] The invention will now be further described by way of examples.

Example 1

[0016] A curable monomer/polymer composition was prepared by mixing poly(ethyl methacrylate) in powdered form (obtained from Bonar Polymers Ltd., Ref. T/S 1249/4, Newton Aycliffe, Co. Durham, U.K. and of molecular weight 250,000) (10g) with tetrahydrofurfuryl methacrylate monomer (obtained from Rohm Chemie, Darmstadt, Germany) (5 ml) containing 2.5% v/v of N,N'-dimethyl-p-toluidine as activator. The polymer component contained 8% w/w BaSO₄ incorporated during the polymerisation process to confer radioopacity.

[0017] Human growth hormone (obtained from Novo Nordisk, Denmark) was incorporated into the material by mixing 12 IU with 10g powder component prior to adding the monomer.

[0018] The composition was cast in discs of diameter 2mm at a temperature of 37°C. Elution of the growth hormone was monitored by immersion of the discs in 0.1M phosphate-buffered saline at 37°C at suitable time intervals using a specific ELISA. Figure 1 shows the *in vitro* release of human growth hormone.

[0019] The surface properties of the discs as cast were examined by scanning electron microscopy. This revealed that the cured polymer had a smooth surface, as compared to the rough surfaces obtained with more conventionally employed polymethyl methacrylate.

[0020] Portions of 2ml of the curable composition prepared as above, immediately after mixing, were inserted by syringe into drilled holes in the knees of three rabbits (adult Sandy Lop, weight of least 3.5kg) which were then kept unrestrained for eight months. Rapid healing and wound closure were noted and the rabbits quickly regained mobility and appetite. The tissue response at the bone-polymer interface and cartilage-polymer interface were examined. At the macroscopic level it was apparent that the cartilage defect had healed.

Example 2

[0021] A curable composition as described in Example 1 was employed. The composition was in the form of a liquid monomer containing the dispersed polymer.

5 [0022] A single 3 mm diameter defect was drilled into the intercondylar notch of the articular cartilage of 18 mature Sandy Lop rabbits. Into each defect was inserted 0.15 ml of the curable composition containing human growth hormone (12 international units per 5g polymer powder) into the subchondral bone below the area of removed cartilage. Plain curable composition without any growth hormone was inserted into a similar cartilage defect in the contralateral limb. Rabbits were sacrificed at 3, 6, 9 and 12 weeks and 8 months.

10 [0023] Histology. At each time interval, excess bone was removed from the femoral condyles before fixation in 2% paraformaldehyde and 0.5% glutaraldehyde, at 4°C, for 48 hours. Each specimen was decalcified in neutral EDTA at 4°C prior to either low temperature (4°C) dehydration and wax embedding, or frozen section preparation.

[0024] Cryosectioning. At each time interval decalcified specimens were frozen in cryomountant using liquid nitrogen. These were cryostat sectioned at -20°C, whilst the polymer was held in place with double-sided sellotape, sections were then mounted on glass slides and stained.

15 [0025] Immunolocalisation of collagen type II, chondroitin 4 sulphate and chondroitin 6 sulphate. Dewaxed sections were chondroitinase digested (0.25 IU/ml) for one hour at 38°C to reveal the epitopes before immunolocalisation. Selected primary monoclonal antibodies were used individually and a rhodamine conjugated anti-murine serum was applied to each section, after appropriate wash steps between stages. Non-immune mouse serum was applied as control to all immunolocalisations and pre-absorbed anti-collagen type II monoclonal was used as an extra control for the localisation of collagen type II which is specific to cartilage.

20 [0026] Electron Microscopy. At each time interval, excess bone was removed from the femoral condyles before fine trimming to the area of defect repair, before fixation with 2.5% glutaraldehyde in sodium cacodylate buffer at 4°C, for a minimum 48 hours. Specimens were post fixed in 1% osmium tetroxide for 2 hours before dehydration through a methanol series into propylene oxide and embedding in Spurr's resin.

25 [0027] The results observed were as follows:

[0028] Macroscopic findings. None of the rabbits died, nor was there any evidence of infection. The rabbits were housed in group pens which allowed freedom of movement i.e. running, jumping, standing on hind legs. The animals showed no sign of discomfort and all enjoyed full mobility.

30 In most rabbits the knee joints showed a white glistening cartilage-like tissue resembling the normal surrounding articular cartilage. There appeared to be a good overgrowth of cartilage over the polymer within the defect. In three rabbit knees the tissue covering was incomplete; histological observations revealed that the polymer had been set above the level of the subchondral bone in the cartilage defect. Since cartilage cannot grow through the polymer, it is therefore important that the polymer is set at the right level to allow good resurfacing.

35 [0029] Two rabbits were kept for a longer study (eight months). The joints remained functional throughout the study period. The histology revealed that the new cartilage remained intact but the density of the matrix had still not achieved that of the original cartilage. There were still a mixed population of cells and areas of fibrous and chondrogenic regions. The subchondral bone had remodelled and in it the polymer became surrounded by very dense collagen.

40 [0030] Frozen sections. Cryostat sectioning allowed visualisation of the intact polymer-tissue interface, hence the extracellular matrix components of the tissue growing over the polymer three weeks after surgery were characterised. Histologically a variety of tissues were observed. Most prominent in the early stages was the observation of a highly cellular fibrous tissue. A thin layer of synovial appearance separated the new tissue covering the polymer from the intracondylar space. Bony spicules appeared to be associated with areas of new tissue immediately adjacent to the polymer surface. Above this interface the fibrous layer contained areas of rounded cells in a metachromatically stained matrix believed to be chondrogenic nodules.

45 [0031] Low temperature wax embedded tissue sections. Immunolocalisation of collagen type II within the cartilaginous nodules confirmed the chondrogenic phenotype of these areas of the tissue. Immunolocalisation studies also demonstrated an elaboration of chondroitin 4-sulphate and chondroitin 6-sulphate glycosaminoglycan side chains both in the fibrous tissue and in the regions of chondrogenic nodules. The varieties of cell phenotypes within the layer covering the polymer were shown histologically and by immunolocalisation of their matrix molecules during the first twelve weeks after implantation.

50 [0032] Transmission electron microscopy of the trans-polymer tissue layer after 8 months of implantation showed the rounded appearance of cells within a proteoglycan rich matrix, indicating the chondrogenic nature of the tissue. The presence of chondron at the cartilage and bone interface was noted.

55 [0033] Growth hormone incorporation. It was shown that the cured polymer system was a good vehicle for the release of growth hormone. Morphological comparisons were made between the tissue covering the growth hormone and plain polymer.

[0034] Figures 2 to 6 illustrate the above findings as follows:

Figure 2

[0035] After 3 weeks of implantation a fibrous tissue layer (f) had grown over the polymer (P) surface. The polymer (P) had been inserted into a sub-chondral defect in the bone (B) below the level of the remaining cartilage (C).

[0036] Decalcified tissue embedded in wax. Section is stained with Methylene blue-Azur II.

Figure 3

[0037] After 6 weeks of implantation two zones of repair tissue were observed. Bony spicules (b) and nodules containing chondrocytes (arrowed) are seen in the zone immediately above the polymer (P) surface. Where the defect has been made in the cartilage (C) a tissue layer similar in appearance but less organised than normal cartilage (c) has formed above the bony zone. Original bone is denoted B, and the original cartilage is denoted C.

[0038] Decalcified tissue embedded in wax. Section is stained with Methylene blue-Azur II.

Figure 4

[0039] After 9 weeks of implantation there is more new bone (b) above the polymer (P) surface. There are nodules containing chondrocytes (arrow) in the bony layer. The new cartilage (c) is disorganised. Original bone is denoted B, and the original cartilage is denoted C.

[0040] Decalcified tissue embedded in wax. Section is stained with Methylene blue-Azur II.

Figure 5

[0041] By transmission electron microscopy clusters of chondrocytes are seen to be contained within a collagenous basket (co). These are referred to as chondrons and are structures normally observed in the deep zones of mammalian cartilage. The normal extracellular matrix is denoted M.

Figure 6

[0042] The cells within the chondron (Co) appear to be actively synthesising cell products indicated by the enormous amount of endoplasmic reticulum (ER). The cell nucleus is denoted N and the normal extracellular matrix is denoted M.

Claims

1. The use of a monomer/polymer mixture in the preparation of a curable composition for introduction at or adjacent to a cartilage site requiring repair in a human or animal body to promote said repair by curing *in situ*, the monomer component being selected from monomeric esters of general formula I



where R is a hydrogen atom or a methyl group, m is 0, 1 or 2 and X is a 3 to 6 membered heterocyclic ring and the polymer component is selected from acrylate and methacrylate polymers and copolymers thereof.

2. A use according to Claim 1, wherein X in the monomer component of formula I is an oxygen-containing heterocycle.
3. A use according to Claim 2, wherein the monomer component is tetrahydrofurfuryl methacrylate.
4. A use according to Claim 1, 2 or 3, wherein the polymer component is poly(ethyl methacrylate).
5. A use according to any one of the preceding claims in the preparation of a curable composition having a polymer to monomer ratio of 1:1 to 2:1 by weight.
6. A use according to any one of the preceding claims, in the preparation of a curable composition further comprising one or more components selected from antibiotic and therapeutic agents, antifungal and antimicrobial agents, porogens, protein carriers and cell growth stimulants.

7. A use according to Claim 6, wherein the, or one of the, additives is human growth hormone.

Patentansprüche

1. Verwendung einer Monomer/Polymer-Mischung zur Herstellung einer härtbaren Zusammensetzung zum Einführen bei oder in Nachbarschaft zu einer Knorpelstelle, die einer Reparatur bedarf, in einem menschlichen oder tierischen Körper, um die Reparatur durch in situ erfolgendes Härten zu fördern, wobei die Monomerkomponente ausgewählt ist aus monomeren Estern der allgemeinen Formel I

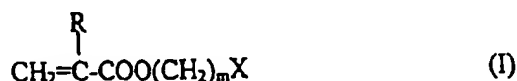


in der R ein Wasserstoffatom oder eine Methylgruppe ist, m 0, 1 oder 2 ist und X ein 3- bis 6-gliedriger heterocyclischer Ring ist und die Polymerkomponente ausgewählt ist aus Acrylat- und Methacrylatpolymeren und Copolymeren davon.

2. Verwendung nach Anspruch 1, wobei in der Monomerkomponente der Formel I X ein Sauerstoff enthaltender Heterocyclus ist.
3. Verwendung nach Anspruch 2, wobei die Monomerkomponente Tetrahydrofurfurylmethacrylat ist.
4. Verwendung nach Anspruch 1, 2 oder 3, wobei die Polymerkomponente Poly(ethylmethacrylat) ist.
5. Verwendung nach einem der vorangehenden Ansprüche zur Herstellung einer härtbaren Zusammensetzung mit einem Gewichtsverhältnis von Polymer zu Monomer von 1:1 bis 2:1.
6. Verwendung nach einem der vorangehenden Ansprüche zur Herstellung einer härtbaren Zusammensetzung, die ferner eine oder mehrere Komponenten, ausgewählt aus antibiotischen und therapeutischen Mitteln, fungiziden und antimikrobiellen Mitteln, Porositätsbildnern, Proteinträgern und Zellwachstumsstimulantien, umfaßt.
7. Verwendung nach Anspruch 6, wobei das oder eines der Additive menschliches Wachstumshormon ist.

Revendications

1. Utilisation d'un mélange monomère/ polymère dans la préparation d'une composition durcissable destinée être introduite à l'emplacement d'un cartilage ou à son voisinage, nécessitant une réparation dans un corps humain ou animal afin de favoriser ladite réparation par durcissement *in situ*, le composant monomère étant choisi parmi les esters monomères de formule générale I



dans laquelle R est un atome d'hydrogène ou un groupe méthyle, m est 0, 1 ou 2 et X est un noyau hétérocyclique à 3 - 6 éléments et le composant polymère est choisi parmi des polymères acrylates et méthacrylates et les copolymères de ceux-ci.

2. Utilisation selon la revendication 1 dans laquelle X, dans le composant monomère de formule I, est un hétérocycle contenant de l'oxygène.
3. Utilisation selon la revendication 2 dans laquelle le composant monomère est le méthacrylate de tétrahydrofurfuryle.

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4. Utilisation selon la revendication 1, 2 ou 3 dans laquelle le composant polymère est le poly (méthacrylate d'éthyle).
5. Utilisation selon l'une quelconque des revendications précédentes dans la préparation d'une composition durcissable ayant un rapport polymère : monomère de 1 :1 à 2 :1 en poids.
- 5 6. Utilisation selon l'une quelconque des revendications précédentes dans la préparation d'une composition durcissable comprenant en outre un ou plusieurs composants choisis parmi les agents antibiotiques et thérapeutiques, les agents antifongiques et antimicrobiens, les porosogènes, les porteurs protéiques et les stimulateurs de croissance cellulaire.
- 10 7. Utilisation selon la revendication 6, dans laquelle les additifs, ou l'un de ceux-ci, est l'hormone de croissance humaine.

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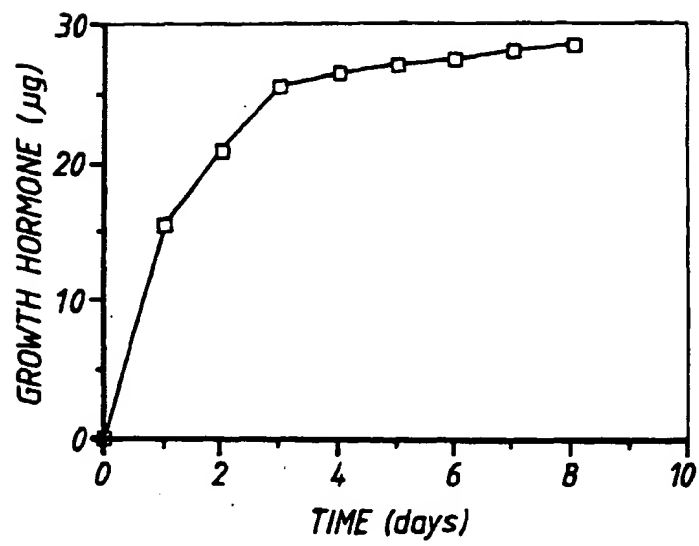


Fig. 1

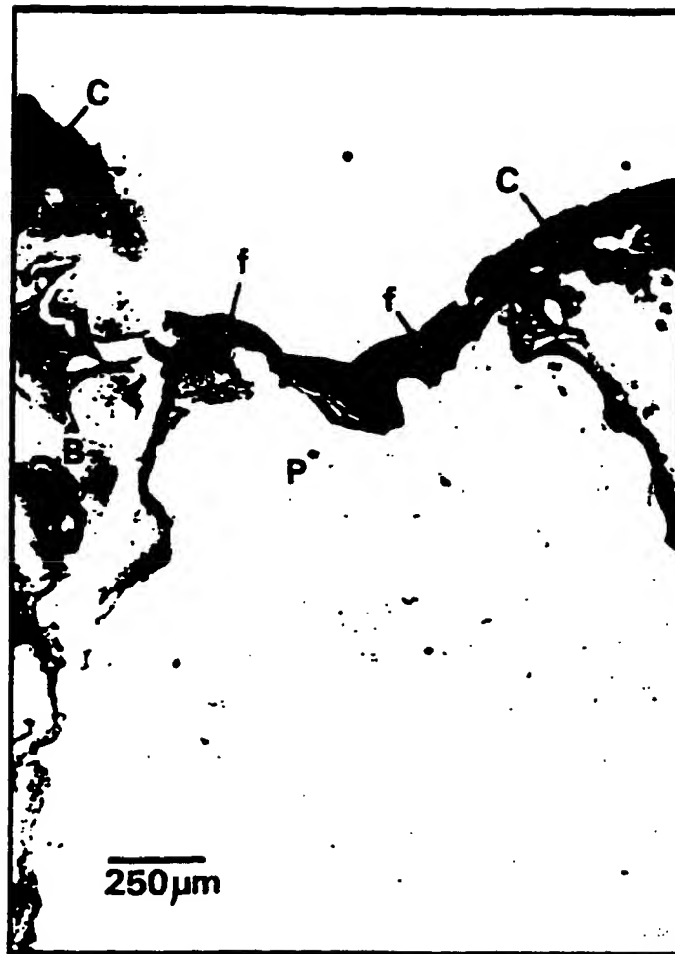


Fig. 2



Fig. 3

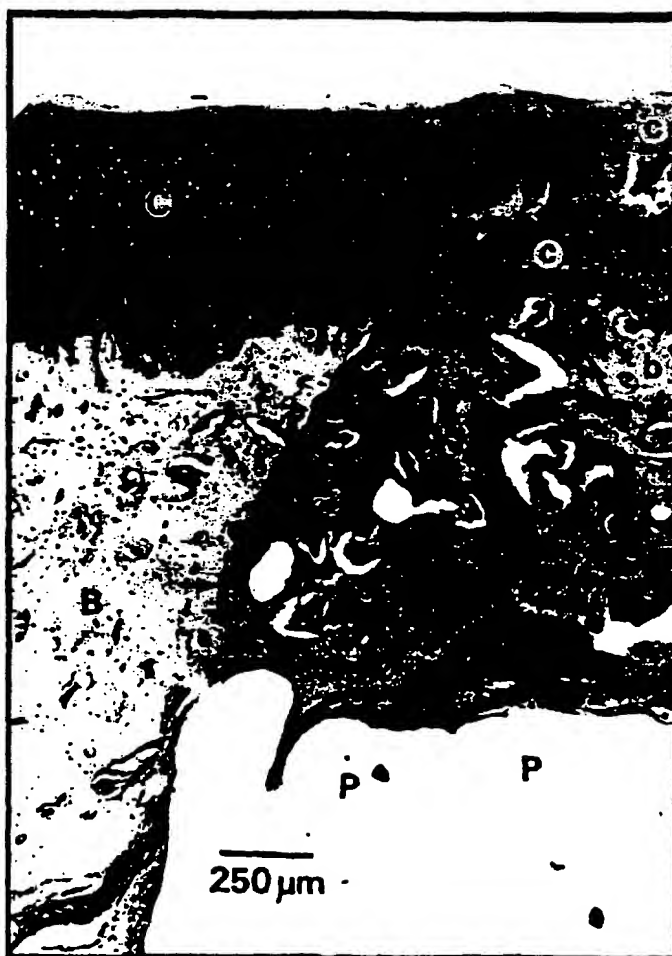


Fig. 4



Fig. 5

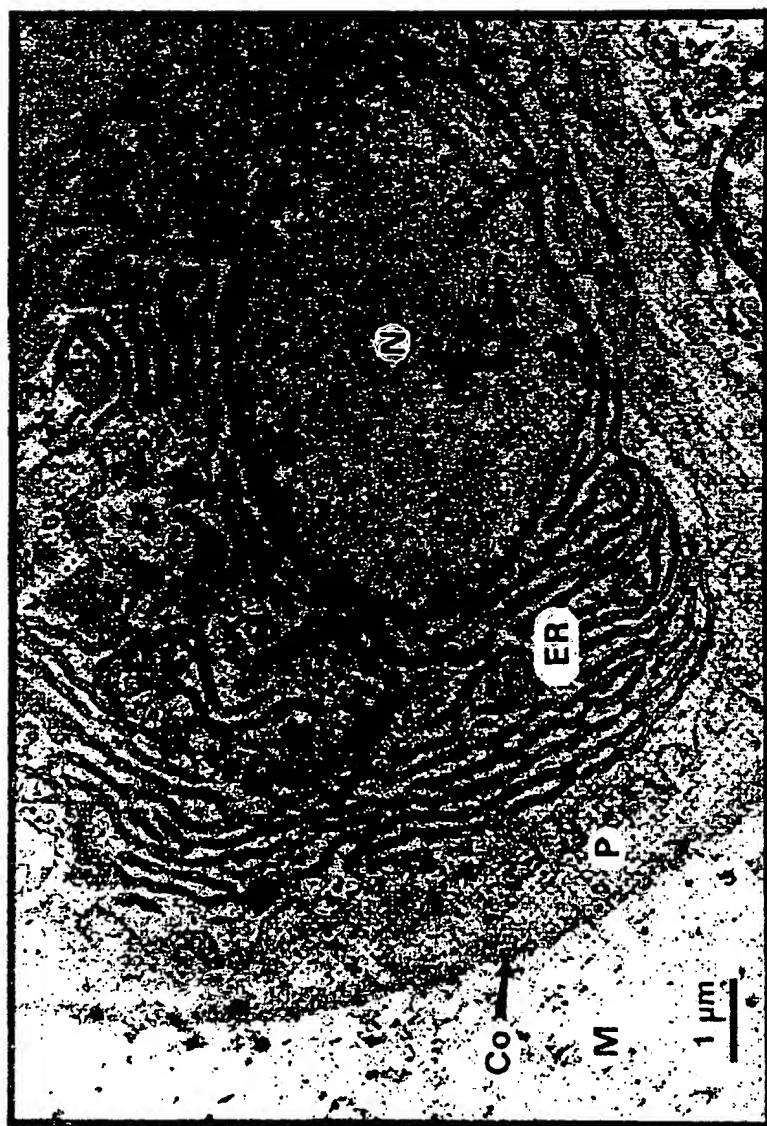


Fig. 6